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RESEARCH ARTICLE

Investigation on the Polymorphism of Some Loci by Using PCR-RFLP in Japanese Quails (*Coturnix coturnix japonica*) Raised in Different Locations of Turkey [1]

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Summary

The aim of this study was to investigate the restriction fragment length polymorphism (RFLP) of some loci on chromosomes 1 (CJA1) and 3 (CJA3) in Japanese quails (*Coturnix coturnix japonica*) raised in Turkey. With this study population genetics parameters were estimated in order to select individuals for establishing a reference population, which would be used for studies on recombination frequency. Fertilized eggs obtained from flocks raised in six different provinces of Turkey were incubated for 120 h and 191 embryos were collected. From the tissue samples of the embryos, DNA was isolated by using DNA isolation kits. Particular regions of *SEMA3E, IFR1, HAL, LOC396025, UGP2, LOC396192, TLX* and *BMP5* loci were amplified with specifically designed primers for each locus by using PCR technique. The PCR products were cut with an appropriate restriction enzyme for each locus and analysed by using agarose gel electrophoresis. Presence of different alleles, allele and genotype frequencies, heterozygosities and genetic distances were estimated. Out of the eight loci studied, polymorphism was found for the *SEMA3E* and *TLX* loci on 1. and 3. chromosomes, respectively, while five loci were found to be monomorphic and one locus (*HAL*) could not be amplified by PCR. The populations studied were found to be mostly in Hardy-Weinberg equilibrium. The results indicated that the *SEMA3E* and *TLX* loci can be used for studying recombination frequencies in the populations included into the study.

Keywords: *Japanese quail, RFLP, Polymorphism, Recombination*

Türkiye'deki Japon Bıldırcınlarında (*Coturnix coturnix japonica*) Bazı Lokuslardaki Polimorfizmin PCR-RFLP Yöntemi İle Araştırılması

Özet

Bu çalışmanın amacı Japon bıldırcınlarında (*Coturnix coturnix japonica*) 1. (CJA1) ve 3. (CJA3) kromozom üzerinde bulunan bazı genlerdeki kesim bölgesi polimorfizminin (RFLP) araştırılmasıdır. Bu çalışma ile rekombinasyon oranları ile ilgili araştırma amacıyla kullanılacak bir popülasyonu oluşturacak bireylerin seçimine temel teşkil edecek olan popülasyon genetiği parametrelerinin ortaya konması amaçlanmıştır. Türkiyenin altı farklı ilindeki işletmelerden elde edilen döllü bıldırcın yumurtaları 37°C'de 120 saat süreyle inkube edildikten sonra 191 adet embriyodan doku örneği alınmıştır. Alınan doku örneklerinden özel kitler yardımıyla DNA izolasyonu yapılmıştır. *SEMA3E, IFR1, HAL, LOC396025, UGP2, LOC396192, TLX* ve *BMP5* lokuslarının belirli bölgeleri özel olarak dizayn edilmiş olan primerler yardımıyla ve PCR işlemi ile çoğaltılmıştır. Elde edilen PCR ürünleri uygun bir restririksiyon enzimi ile kesilmiş ve agaroz jel elektroforezi yardımıyla ayrımlanmıştır. İncelenen popülasyonlarda farklı allellerin varlığı, genotip ve allel frekansları, heterozigotluk dereceleri ve genetik mesafeler hesaplanmıştır. Çalışmada kullanılan sekiz lokustan 1. ve 3. kromozomlar üzerinde bulunan *SEMA3E* ve *TLX* lokuslarında polimorfizm tespit edilmiş, beş lokus monomorfik olarak bulunmuş bir lokusta (*HAL*) ise PCR işlemi ile çoğaltma yapılamamıştır. Çalışılan popülasyonların büyük oranda Hardy-Weinberg dengesinde oldukları gözlenmiştir. Araştırmanın sonuçları *SEMA3E* ve *TLX* lokuslarının çalışılan popülasyonlarda rekombinasyon oranlarının araştırılması amacıyla kullanılabileceğini göstermiştir.

Anahtar sözcükler: *Japon bıldırcını, RFLP, Polimorfizm, Rekombinasyon*

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INTRODUCTION

Recombination due to cross over is controlled by many enzyme and factors [1]. Recombination frequency between two loci on the same chromosome varies depending on the distance between two loci considered, as well as between sexes or families [2-4].

In some cases, it may be necessary that an allele of a locus is linked to a particular allele of another locus on the same homologous chromosome in coupling phase. This can only be achieved via recombination, if the alleles considered are on different homologous chromosomes in repulsion phase. On the other hand, the probability of recombination between closely linked loci is very low. In this case it takes a long time or a large number of individuals should be examined, in order to obtain a particular combination of alleles linked on the same chromosome. However some drugs may elevate recombination rate. Kunz et al.[5] have shown that 5-floro-deoxy uridinmonophosphate (FdUMP) increase mitotic recombination in yeast *(Saccharomyces cereviciae)* by inhibiting the enzyme thimidine synthase. Silber et al.[6] have reported that fluorodeoxyuridine (FUdR) and aminopterine elevates meiotic recombination in *Drosophyla melanogaster*, by inhibiting dihydrofolate reductase enzyme.

In order to estimate recombination frequency it is necessary to know whether an allele is of maternal or paternal origin. The most convenient way for this is back crossing of F1 individuals, which have been obtained by crossing two different homozygous lines with one of the parental lines. Therefore polymorphic loci and homozygous lines for the loci considered are necessary.

Japanese quail *(Coturnix coturnix japonica)* is an ideal experimental animal species due to its small body size, lower cost of care and high reproduction rate. Therefore Japanese quails have been widely used for studies of different purposes [7-9]. Since Japanese quail is closely related to chickens, it is an ideal model organism as well [10,11].

The aim of this study was to investigate the variability of eight loci located on chromosomes 1 (CJA1) and 3 (CJA3) of Japanese quails raised in different provinces of Turkey by using polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) method. It was aimed to estimate some population genetics parameters in order to select individuals for establishing a reference population, which would be used for studies on recombination frequency.

MATERIAL and METHODS

Sample Collection and DNA Isolation

Fertilized Japanese quail eggs were purchased from six different provinces of Turkey, and incubated at 37°C and 70% relative humidity for 120 h. Following the incubation the eggs were stored at 4°C for 24 h, and the embryos were taken into microfuge tubes containig 96% ethanol and stored at -20°C until DNA isolation. A total of 191 embryos were obtained from Gaziantep (n= 40), Konya (n= 33), Manisa (n= 16), Mersin (n= 23), Afyon (n= 39) and Eskişehir (n= 40) provinces. From the embryos, DNA was isolated by using DNA isolation kits according to instructions of the manufacturer (Fermentas, Vilnius, Lithuania).

Selection of the loci and Genotyping of the Samples

The loci included into the study and sequences of the primers used for polymerase chain reaction (PCR) were shown in *Table 1*. The loci included into the study were selected based on the chromosome map constructed by Sasazaki et al.[11]. The primers used for amplification of the loci were designed based on the sequences submitted to the GenBank by Sasazaki et al.[11], by using Primer-Blast program *(http://www.ncbi.nlm.nih.gov)*.

PCR was carried out in 25 µL reaction volume. The reaction mix consisted of 0.4 µM of each primers, 200 µM dNTPs each, 2 mM $MgCl_2$, 1.25 U DNA polymerase (Thermo Scientific, Espoo, Finland) and 2.5 µL of 10X reaction buffer containing 10 mM Tris-HCl (pH 8.8), 50 mM KCl and 0.1% Triton X-100. The amplification protocol consisted of an initial denaturing step of 94°C for 5 min, followed by 10 cycles of 94°C for 30 s, 60°C for 30 s decreasing 1°C in each cycle and 72°C for 30 s and 25 cycles of 94°C for 30 s, 50°C for 30 s, 72°C for 30 s. In the final cycle the extension step was carried out at 72°C for 10 min

A 10 µL of PCR product was digested by using the restriction enzyme reported by Sasazaki et al.[11] according to the instructions of the manufacturer (Fermentas, Vilnius, Lithuania). The restriction enzymes and reaction conditions used were shown in *Table 2*. Control of the PCR and digestion products was performed by electrophoresis on a 2% agarose gel stained with ethidium bromide.

Data Analysis

Observed and expected genotype frequencies were compared by chi-square test [12]. Inbreeding coefficients (F-values) in each population and genetic distances due to inbreeding were estimated according to Weir and Cockerham [13]. Genetic distances between the populations were estimated according to Nei's formula (D_{SA}) [14]. Significance of the differentiation between populations was tested according to Raymond and Rousset [15]. Software packages of GENEPOP Version 3.1 [16], GenAlEx.6 [17], ARLEQUIN 3.5 [18] and POPGENE 1.31 [19] were used for the analysis of the data.

The study was carried out with the permission of Harran University Animal Experimentation Local Ethics Committee [Approval No: 2010/04 (1/2)].

Table 1. *The loci included into the study and sequences of the primers used.*
Tablo 1. *Araştırmaya dahil edilen lokuslar ve kullanılan primerlerin baz dizisi*

Locus	Chromosomal Location (cM)	*Primer Sequence (5'-3')*	Accession Number
SEMA3E	CJA1 (70.5)	Forward-ATACTCCAGCTGAGTGGGGA	AB250305
		Reverse-CAGAAGTATGAGGGAGATCAG	
IFR1	CJA1 (88.8)	Forward-AGTGTGCAGCCTTTTAGTGATGAAG	AB250306
		Reverse-TGAAGGGAGGCTGTAGTGAG	
HAL	CJA1 (106.8)	Forward-AATCACCACAGGCTTTGGGA	AB250307
		Reverse-TTCCACTGTAGCCCTTTGCG	
UGP2	CJA3 (12.5)	Forward-TTGGTGTGTGTGCTTCAGAG	AB250322
		Reverse-CCAGTCTGCATTGCCTAAC	
LOC396025	CJA3 (24.6)	Forward-TGGTGACCAGCACCAAAGC	AB250321
		Reverse-TTCCACTGTAGCCCTTTGCG	
LOC396192	CJA3 (157.4)	Forward-AGTGGTTATTGCCTGTGGTT	AB250325
		Reverse-AGGAGTAGTAAGTGAAGCCTG	
TLX	CJA3 (165.4)	Forward-ACACTAGGAACATAATGGGCT	AB250326
		Reverse-TCACTGTGGCGTTTCAGATT	
BMP5	CJA3 (171.6)	Forward-ACTGATCATAAGCGTGCCCT	AB250327
		Reverse-CCAGACGCTTACACTGTGC	

Table 2. *Restriction enzymes and reaction conditions used for each loci*
Tablo 2. *Restriksiyon enzimleri ve her bir lokus için kullanılan reaksiyon şartları*

Locus	Enzyme	Amount (Unit)	Incubation (°C/h)	Inactivation	Buffer
SEMA3E	*Hae* III	5	37/12	80°C /20 min.	R
IFR1	*Hin*6I	5	37/12	65°C/ 20 min.	Tango
HAL	*Nco*I	5	37/12	65°C/ 20 min.	Tango
LOC396025	*Mse*I	2.5	65/12	0.5 M EDTA	R
UGP2	*Hpa*I	5	37/12	65°C/ 20 min.	B
LOC396192	*Mse*I	2.5	65/12	0.5 M EDTA	R
TLX	*Pst*I	2.5	37/12	0.5 M EDTA	O
BMP5	*Tai*I	5	65/12	0.5 M EDTA	R

RESULTS

The loci included into the study, except for the *HAL* locus, were successfully amplified by using the primer pairs designed. Length of the PCR products of different loci varied from 356 to 630 *(Table 3)*. After cutting with the appropriate enzymes, one to five different fragments for each locus were observed *(Table 3)*. Restriction products of some samples for each locus were shown in *Fig 1*. Polymorphism was observed only for the *SEMA3E* and *TLX* loci. For the *SEMA3E* locus three different alleles and four genotypes were observed, while two different alleles and three genotypes were found for the *TLX* locus. Allele and genotype frequencies of *SEMA3E* and *TLX* loci in each population were shown in *Table 4* and *5*, respectively.

Number of alleles, heterozygosities and inbreeding coefficients of *SEMA3E* and *TLX* loci in each population were given in *Table 6*. Deviation from the equilibrium was observed only for *TLX* locus in Manisa population ($P<0.05$). When the *SEMA3E* and *TLX* loci were considered together, significant genotypic differentiations were observed

Table 3. *Fragment lengths of PCR and restriction products.*
Tablo 3. *PCR ve restriksiyon ürünlerinin fragman uzunlukları*

Locus	Length of PCR Product (bp)	Cut	Lengths of Restriction Fragments (bp)
SEMA3E	412	+	412, 362+50, 335+77
IFR1	630	+	390+240
HAL	-	-	-
LOC396025	385	+	252+83+50
UGP2	476	-	476
LOC396192	363	+	264+78+21
TLX	546	+	546, 404+142
BMP5	356	+	281+75

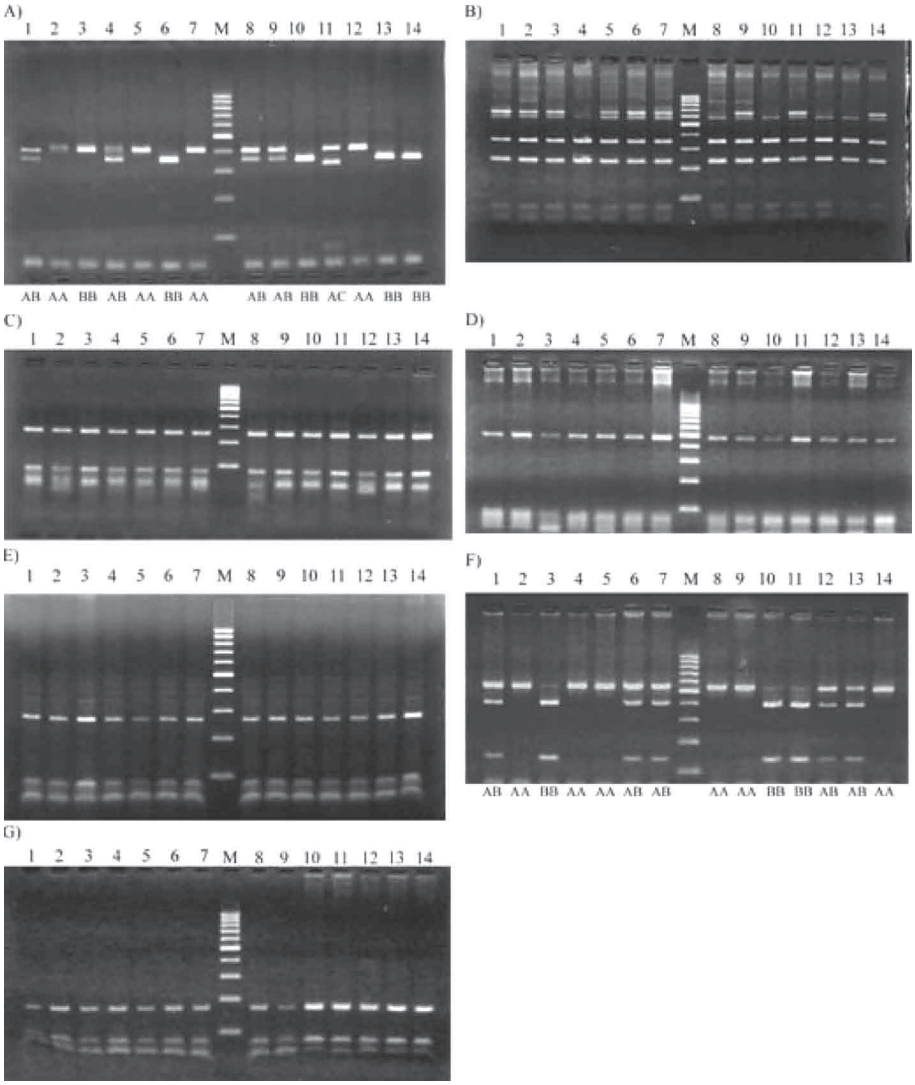

Fig 1. Restriction products of some samples on 2% agarose gel. **A)** *SEMA3E*; **B)** *IFRI*; **C)** *LOC396025*; **D)** *UPG2*; **E)** *LOC396192*; **F)** *TLX*; **G)** *BMP5*. Lines 1-14: Samples; M: Molecular size standard (100 bp ladder). The genotypes of the samples assigned for *SEMA3E* and *TLX* loci were shown at the bottom of the related picture

Şekil 1. Bazı örneklere ait restriksiyon ürünlerinin %2'lik agaroz jeldeki görünümü. **A)** *SEMA3E*; **B)** *IFRI*; **C)** *LOC396025*; **D)** *UPG2*; **E)** *LOC396192*; **F)** *TLX*; **G)** *BMP5*. 1-14: Örnekler; M: Moleküler standart (100 bç merdiven). Örneklerin *SEMA3E* ve *TLX* lokusları açısından genotipleri ilgili resmin altında gösterilmiştir

Table 4. *Allele frequencies at the SEMA3E and TLX loci in each population*
Tablo 4. *Her bir populasyondaki SEMA3E ve TLX lokuslarına ait allel frekansları*

Locus	Allel	Gaziantep (n=40)	Mersin (n=23)	Konya (n=33)	Manisa (n=16)	Eskişehir (n=40)	Afyon (n=39)	All (N=191)
SEMA3E	A	0.625	0.674	0.652	0.469	0.738	0.769	0.675
	B	0.363	0.326	0.318	0.531	0.263	0.231	0.317
	C	0.013	0.000	0.030	0.000	0.000	0.000	0.008
TLX	A	0.550	0.391	0.606	0.563	0.488	0.654	0.550
	B	0.450	0.609	0.394	0.438	0.513	0.346	0.450

between Mersin and Afyon ($P<0.05$), Manisa and Eskişehir ($P<0.05$) as well as Afyon ($P<0.01$) populations. The lowest genetic distance was observed between Gaziantep and Konya populations, while the highest genetic distance was observed between Manisa and Afyon populations.

DISCUSSION

The loci in this study were selected due to their location on the chromosomes, by considering the map reported by Sasazaki et al.[11]. The loci should be closely linked to each other so that a recombination could be detected and double recombinations did not occur at a high frequency. In addition the loci *SEMA3E, HAL* and *IFR1* on CJA1 as well as *LOC396025, TLX* and *BMP5* on CJA3 were selected, in order to assess an interference between the loci. The functional properties of the loci were not considered.

All the loci, except for *HAL* locus, were successfully amplified by using the designed primer pairs and PCR method. Except for the *UGP2* locus, PCR products of *IFR1*,

Table 5. *Genotype frequencies at the SEMA3E and TLX loci in each population*
Tablo 5. *Her bir populasyonda SEMA3E ve TLX lokuslarına ait genotip frekansları*

Locus	Genotype	Gaziantep	Mersin	Konya	Manisa	Eskişehir	Afyon	All
SEMA3E	AA	0.325	0.522	0.424	0.125	0.600	0.564	0.450
	AB	0.575	0.304	0.394	0.687	0.275	0.410	0.424
	AC	0.025	0.000	0.061	0.000	0.000	0.000	0.016
	BB	0.075	0.174	0.121	0.188	0.125	0.026	0.110
	BC	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	CC	0.000	0.000	0.000	0.000	0.000	0.000	0.000
TLX	AA	0.275	0.174	0.424	0.438	0.250	0.410	0.320
	AB	0.550	0.435	0.364	0.250	0.475	0.487	0.450
	BB	0.175	0.391	0.212	0.312	0.275	0.103	0.230

Table 6. *Number of alleles, heterozygosities and inbreeding coefficients for SEMA3E and TLX loci in each population*
Tablo 6. *Her bir populasyonda SEMA3E ve TLX lokuslarına ait allel sayıları, heterozigotluk derecesi ve kanyakınlı/ğı katsayısı*

Population	Locus	Na	Ho	He	UHe	F
Gaziantep	*SEMA3E*	3	0.600	0.478	0.484	-0.256
	TLX	2	0.550	0.495	0.501	-0.111
Mersin	*SEMA3E*	2	0.304	0.440	0.449	0.308
	TLX	2	0.435	0.476	0.487	0.087
Konya	*SEMA3E*	3	0.455	0.473	0.481	0.040
	TLX	2	0.364	0.478	0.485	0.238
Manisa	*SEMA3E*	2	0.688	0.498	0.514	-0.380
	TLX	2	0.250	0.492	0.508	0.492
Eskişehir	*SEMA3E*	2	0.275	0.387	0.392	0.290
	TLX	2	0.475	0.500	0.506	0.049
Afyon	*SEMA3E*	2	0.410	0.355	0.360	-0.156
	TLX	2	0.487	0.453	0.459	-0.076
All	*SEMA3E*	3	0.440	0.443	0.445	0.008
	TLX	2	0.450	0.495	0.496	0.090

Na: *Number of alleles;* ***Ho:*** *Observed heterozygosity;* ***He:*** *Expected heterozygosity;* ***UHe:*** *Unbiased expected heterozygosity;* ***F:*** *Inbreeding coefficient*

LOC396025, *LOC396192*, *BMP5*, *SEMA3E* and *TLX* loci were cut with the restriction enzymes *(Table 3)* at specific sites. PCR products of *UGP2* locus were not cutable with *Hpa*I. This indicated that there was no restriction site for this enzyme on target region.

When the PCR products of the *IFR1*, *LOC396025*, *LOC39619* and *BMP5* loci were cut with the respective enzyme, specific banding patterns for each locus were observed (*Table 3* and *Fig. 1B, C, D, E* and *G,* respectively). However no variability between individuals for the banding patterns of these loci was observed. Therefore the data for these loci were not included into the estimation of population genetics parameters.

When restriction products of *SEMA3E* locus was examined five different bands were observed, suggesting the presence of two polymorphic restriction sites on different position of the PCR products (*Table 3* and *Fig. 1A*). On the other hand three different bands were observed for *TLX* locus (*Table 3* and *Fig. 1F*), indicating the presence of a polymorphic restriction site for *Pst*I enzyme.

To our knowledge there was no report on the polymorphism of the loci used in the present study in other populations of Japanese quails. Although Sasazaki et al.[11] have mapped these loci on the CJA1 and CJA3, they have reported no data on allele frequencies or heterozygosities. Therefore the results of the present study were compared with those reported on other loci or species.

Various genetic markers, such as microsatellites [7,20,21], amplified fragment length polymorphism (AFLP) [22,23] or PCR and restriction fragment length polymorphism (RFLP) [11] have been used to establish a linkage map in Japanese quails. Since by using PCR-RFLP method the presence or absence of a restriction site is detected, two alleles are expected at a particular site [24-26]. However, if there are two

polymorphic sites within the target region, more than two alleles can be found [27-29].

Allele frequencies in various studies, in which the same method has been used as in this study, have varied among populations from 0.000 to 1.000 [26,27]. Therefore allele frequencies observed in this study was in accordance with those reported in the literature.

A deviation from Hardy-Weinberg equilibrium was observed only in Manisa population for *TLX* locus ($P<0.05$). The deviation was due to an excess of homozygotes, as could be seen from the F value. On the other hand, an excess of heterozyotes was found for *SEMA3E*. This suggested that the deviation from the equilibrium might be due to the small sample size from this population.

In order to study recombination frequencies, test individuals should be heterozygous for the two loci considered, which should be polymorphic as well. In this study only *SEMA3E* and *TLX* loci located on CJA1 and CJA3, respectively, were polymorphic and thereby suitable for studying recombination frequency in the Japanese quail populations studied. Therefore further loci or other marker systems, such as microsatellites will be necessary. Individuals, which will be selected for establishing a reference population, should be obtained from the genetically more distant populations, such as Manisa and Afyon populations.

REFERENCES

1. Watson JD, Baker TA, Bell SP, Gann A, Levine M, Losick R: Molecular biology of the Gene. CSHL Press, USA, 2004.

2. Griffiths AJF, Miller JH, Suzuki DT, Lewontin RC, Gelbart WM: An Introduction to Genetic Analysis. W.H. Freeman and Co. New York, 1996.

3. Beeckmann P, Moser G, Bartenschlager H, Reiner G, Geldermann H: Linkage and QTL mapping for Sus scrofa chromosome 8. *J Anim Breed Genet*, 120, 66-73, 2003.

4. Geldermann H, Muller E, Moser G, Reiner G, Bartenschlager H, Cepica S, Stratil A, Kuryl J, Moran C, Davoli R, Brunsch C: Genome-wide linkage and QTL mapping in porcine F2 families generated from Pietrain, Meishan and Wild Boar crosses. *J Anim Breed Genet*, 120, 363-393, 2003.

5. Kunz BA, Barclay BJ, Game JC, Little JG, Haynes H: Induction of mitotic recombination in yeast by starvation for thymine nucleotides. *P Natl Acad Sci USA*, 77, 6057-6061, 1980.

6. Silber J, Bazin C, Lemenn A: Inhibitors of thymidylate synthesis increase whereas thymidine decreases meiotic recombinatino in *Drosphila melanogaster*. *Mol Genet Genomics*, 199, 53-54, 1985.

7. Kayang BB, Vignal A, Inoue-Murayama M, Miva M, Monvoisin JL, Ito S: A first generation microsatellite linkage map of the Japanese quail. *Anim Genet*, 35, 195-200, 2004.

8. Ipek H, Avci M, Yerturk M, Iriadam M, Aydilek N: Effects of ascorbic acid and vitamin E on performance and hematological parameters of Japanese quails under high ambient temperature in Sanliurfa. *Arch Geflügelkd*, 71, 130-134, 2007.

9. Taziaux M, Keller M, Ball GF, Balthazart J: Site-specific effects of anosmia and cloacal gland anestesia on Fos expression induced in male quail brain by sexual behavior. *Behav Brain Res*, 194, 52-65, 2008.

10. Shibusawa M, Minai S, Nishida-Umehara C, Suzuki T, Mano T, Yamada K, Namikawa T, Matsuda Y: A comparative study of chromosome homology between chicken and Japanese quail. *Cytogenet Cell Genet*, 95, 103-109, 2001.

11. Sasazaki S, Hinenoya T, Lin B, Fujiwara A, Mannen H: A comparative map of macrochromosomes between chicken and japanese quail based on orthologous genes. *Anim Genet*, 37, 316-320, 2006.

12. Guo SW, Thompson EA: Performing the exact test of Hardy-Weinberg proportion for multiple alleles. *Biometrics*, 48, 361-372, 1992.

13. Weir BS, Cockerham CC: Estimating F-statistics for the analysis of population structure. *Evolution*, 38, 1358-1370, 1984.

14. Nei M: Genetic distance between populations. *Am Nat*, 106, 283-392, 1972.

15. Raymond M, Rousset F: An exact test for population differentiation. *Evolution*, 49, 1280-1283, 1995.

16. Raymond M, Rousset F: Genepop (Version 1.2) Population genetics software for exact tests and ecumenism. *J Heredity*, 86, 248-249, 1995.

17. Peakall R, Smouse PE: GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. *Mol Ecol Notes*, 6, 288-295, 2006.

18. Excoffier L, Lischer HEL: Arlequin suite ver 3.5: A new series of programs to perform population genetics analyses under Linux and Windows. *Mol Ecol Resour*, 10, 564-567, 2010.

19. Yeh FC, Yang RC, Boyle T, Ye ZH, Mao JX: POPGENE, the user-friendly shareware for population genetic analysis. Molecular Biology and Biotechnology Centre, University of Alberta, Canada, 1997.

20. Minvielle F, Kayang BB, Inoue-Murayama M, Miwa M, Vignal A, Gourichon D, Neau A, Monvoisin JL, Ito S: Microsatellite mapping of QTL affecting growth, feed consumption, egg production, tonic immobility and body temperature of Japanese quail. *BMC Genomics*, 6, 87, 2005, DOI: 10.1186/1471-2164-6-87

21. Minvielle F, Kayang BB, Inoue-Murayama M, Miwa M, Vignal A, Gourichon D, Neau A, Monvoisin JL, Ito S: Search for QTL affecting the shape of the egg laying curve of the Japanese quail. *BMC Genetics*, 7, 26, 2006, DOI:10.1186/1471-2156-7-26

22. Roussot O, Feve K, Plisson-Petit F, Pitel F, Faure JM, Beaumont C, Vignal A: AFLP linkage map of the Japanese quail *(Coturnix japonica). Genet Sel Evol*, 35, 559-572, 2003.

23. Kikuchi S, Fujima D, Sasazaki S, Tsuji S, Mizutani M, Fujiwara A, Mannen H: Construction of a genetic linkage map of Japanese quail *(Coturnix japonica)* based on AFLP and microsatellite markers. *Anim Genet*, 36, 227-231, 2005.

24. Niwa T, Shibusawa M, Natsuda Y, Terashima A, Nakamura A, Shiojiri N: The Bh (Black at Hatch) gene that causes abnormal feather pigmentation maps to chromosome 1 of the Japanese quail. *Pigm Cell Res*, 16, 656-661, 2003.

25. Smith EJ, Geng T, Long E, Pierson FW, Sponenberg DP, Larson C, Gogal R: Molecula analysis of the relatedness of five domesticated turkey strains. *Biochem Genet,* 43, 35-47, 2005.

26. Moe HH, Shimogiri T, Kamihiraguma W, Isobe H, Kawabe K, Okamoto S, Minvielle F, Maeda Y: Analysis of polymorphisms in the insulin-like growth factor 1 receptor (IGF1R) gene from Japanese quail selected for body weight. *Anim Genet*, 38, 659-661, 2007.

27. Okumura F, Shimogiri T, Kotaru K, Okamoto S, Nishibori M, Yamamoto Y, Maeda Y: Gene constitution of South-East Asian native cahickens, commercial chickens and jungle fowl using polymorphisms of four calpain genes. *Anim Sci J*, 77, 188-195, 2006.

28. Sharma P, Bottje W, Okimoto R: Polymorphisms in uncoupling protein, melanocortin 3 receptor, melanocortin 4 receptor, and pro-opiomelanocortin genes and association with production traits in a commercial broiler line 1. *Poultry Sci*, 87, 2073-2086, 2008.

29. Öztabak K, Toker NY, Ün C, Akış I: Leptin gene polymorphisms in native Turkish cattle breeds. *Kafkas Univ Vet Fak Derg*, 16 (6): 921-924, 2010.